REMARKS

Applicants have carefully studied the Office Action mailed on August 12, 2003, which issued in connection with the above-identified application. The present amendments and remarks are intended to be fully responsive to all points of rejection raised by the Examiner and are believed to place the claims in condition for allowance. Favorable reconsideration and allowance of the present claims are respectfully requested.

Applicants gratefully acknowledge the courtesy shown by the Examiner and the Examiner's Supervisor, Jeffrey Stucker, in providing recommendations for response to the present Office Action in an in-person interview with Dr. Erich Hoffmann (the sole inventor), Mr. Jonathan Klein-Evans and applicant's representative, Dr. Paul Fehlner, on December 4, 2003. The Examiner's Interview Summary is attached herein as Exhibit A. This amendment addresses the substantive issues discussed at the interview.

Pending Claims

Claims 2-32, 39 and 42-45 were pending and at issue in the application[1]. Claims 2-14 and 42-43 have been temporarily withdrawn from consideration, but, as specified at page 2 of the Office Action, could be rejoined with claims under examination upon determination of the allowable subject matter. Claims 15-32, 39 and 44-45 have been rejected under 35 U.S.C. § 112, first and second paragraphs, for lack of enablement and as being indefinite. Claims 15-32, 39 and 44-45 have been also rejected under 35 U.S.C. § 102(a) and 103(a) as being anticipated by and/or obvious over the prior art.

[1] Applicants respectfully note that, in the Office Action, the Examiner mistakenly states that claim 1 is pending. Claim 1 has been canceled in the Applicants' Amendment and Response to the Office Action of August 9, 2002.

Claims 6, 18 and 30-31 have been canceled without prejudice or disclaimer. Claims 5, 7, 9, 15-17, 19-29, 39, and 42-45 have been amended to correct formal defects and as suggested by the Examiner in the Office Action and during the in-person interview. No new subject matter has been added as a result of these amendments, no new search is required, and no new issues are raised. Upon entry of these amendments, claims 2-5, 7-17, 19-29, 32, 39, and 42-45 will be pending.

35 U.S.C. §112, First Paragraph, Rejections

In the Action, the Examiner has maintained the rejection of claims 15-32, 39 and 44-45 under 35 U.S.C. §112, first paragraph, for lack of enablement of plasmid-based systems for production of any negative-strand RNA viruses other than influenza viruses. As claims 18 and 30-31 have been canceled, the rejection of these claims is rendered moot. As the remaining claims have been amended to recite only influenza viruses, their rejection is also rendered moot. However, applicants note for the record that this amendment is made solely to expedite the prosecution and not as an admission of non-enablement of the invention for other segmented, negative strand RNA viruses. Applicants reserve the right to pursue this subject matter in a continuing application.

35 U.S.C. §112, Second Paragraph, Rejections

The Examiner has also rejected claims 15-32, 39 and 44-45 under 35 U.S.C. §112, second paragraph, as being indefinite for lack of method steps in claims 15-28 and 44-45. As claims 18 and 30-31 have been canceled, the rejection of these claims is rendered moot. With respect to the remaining claims, applicants respectfully note that claims 15-17, 19-28 and 44-45

are product claims (properly within the elected group). Accordingly, they do not require the recitation of any method steps. Specifically, claims 15-17, 19-24 and 44-45 are directed to a set of plasmids and claims 25-28 are directed to host cells comprising such plasmids. To clarify this further, pursuant the Examiner's suggestion, claims 15-17, 19-28 and 44-45 have been amended to substitute the recitations "minimum plasmid-based system", "plasmid-based system" and "system" with the term "composition". As acknowledged by the Examiner at page 8 of the Office Action, claims 29 and 32 are drawn to methods comprising the use of the host cells of claims 25 and 28, respectively, and claim 39 is drawn to a method comprising the use of the plasmids of claim 15.

In light of the foregoing, applicants respectfully submit that the rejection of the claims based upon 35 U.S.C. §112, second paragraph, is overcome and withdrawal of such is kindly requested.

35 U.S.C. §102(a) Rejections

In the Action, claims 15-32 and 39 stand rejected under 35 U.S.C. §102(a) as being anticipated by Hoffmann *et al.* (Virology, 2000, 267:310-7). As follows from the attached Declaration under 37 C.F.R. §1.132 (*In re Katz*) by the present inventor, Dr. Erich Hoffmann, all other co-authors on the Hoffmann *et al.* publication did not contribute to conception of any of the claimed subject matter and therefore the reference cannot be considered as work "by others" as required under 35 U.S.C. §102(a). Accordingly, the rejection of the claims based upon 35 U.S.C. §102(a) is overcome and withdrawal of such is kindly requested.

35 U.S.C. §103(a) Rejections

In the Office Action, the Examiner has maintained the rejection of claims 15-32, 39 and 44 under 35 U.S.C. §103(a) as being obvious over Hoffmann dissertation (1997) and Neumann *et al.* (Proc. Natl. Acad. Sci., 1999, 96: 9345-50) and has rejected claim 45 as being obvious over Hoffmann and Neumann *et al.* and further in view of Pleshka *et al.* (J. Virol., 1996, 70:4188-92). The Examiner contends that (i) functional elements of the promoters used by Neumann *et al.* are pol II to make mRNA and pol I to drive expression of genomic RNA on different plasmids; (ii) the Hoffmann dissertation teaches a plasmid that contains both promoters, pol I and pol II, that can act as a template for synthesis of both mRNA and genomic RNA; and (iii) Pekosz teaches the complexity of transfection of up to 17 plasmids and the benefit of no helper virus. In light of these contentions, the Examiner submits that one of skill in the art would desire a helper-free system that has fewer plasmids but maintains the viral production efficiency of the Neumann *et al.* reference (with 17 plasmids). The Examiner further contends that the CAT plasmid disclosed in the Hoffmann dissertation does not require a helper virus, because the plasmid was able to make CAT mRNA and then protein in the absence of a helper virus and has the correct ends to be replicated and packaged in the virion. The Examiner also states that Pleschka teaches that ribozymes can be used to generate specific ends of influenza segments. Thus it would allegedly have been *prima facie* obvious to substitute a ribozyme with a terminator in a plasmid used for the production of influenza vRNA with the expectation of success.

As claims 18 and 30-31 have been canceled, the rejection of these claims is rendered moot. With respect to the remaining claims, the rejection is respectfully traversed for the reasons provided below.

Neither of the cited references contains any suggestion or motivation to produce a set of "ambisense" pol I-pol II plasmids capable of generating an infectious influenza virus in the absence of any helper virus or additional protein expression plasmids

Applicants respectfully note that the gist of the present invention as recited in the present claims is the provision of a set of plasmids (each plasmid being an "ambisense" pol I-pol II plasmid comprising one influenza viral genomic segment) which, when co-introduced into a host cell in the absence of any helper virus or additional viral protein-expressing plasmids, *produce an infectious influenza virus*. In other words, there are two critical features of the plasmid composition of the present invention: (1) the total number of plasmids does not exceed the total number of gene segments from the source virus (*e.g.*, 8 plasmids for an 8-segmented influenza A virus), and (2) these plasmids can reconstitute an infectious virus in the absence of any helper virus or additional viral protein-expressing plasmids.

While Neumann *et al.* article achieves the goal of reconstituting an infectious influenza virus in the absence of a helper virus, in contrast to the present invention, it does so using a totally different set of plasmids, which lack any "ambisense" pol I-pol II plasmids. Instead, the Neumann *et al.* system contains pol I-only plasmids directing synthesis of vRNAs from a pol I promoter and pol II-only protein expression plasmids encoding viral polymerase proteins, making the total number of plasmids exceed the total number of gene segments from the source virus (*e.g.*, 12 to17 plasmids for an 8-segmented influenza A virus).

Also, as admitted by the Examiner in the previous Office Action, Neumann *et al.* teach that increasing the number of different co-transfected protein expression plasmids from 4 to 9 increases the resulting viral titer (see, p. 9347 [bridging left and right col.] and Table 1 at p. 9348). Based on this disclosure, a person skilled in the art would be persuaded to increase, not to decrease, the number of expression plasmids. Thus, as stated at p. 8806 of the review by Pekosz *et al.* cited by the Examiner (left col., ¶2): "The number of recombinant viruses rescued can be

increased nearly 10-fold by including plasmids encoding the hemagglutinin, NA, M1, M2, and NS2 proteins under control of the pol II promoter in the transfection."[2] Thus, Neumann *et al.* reference teaches that an efficient viral rescue requires more plasmids.

The Hoffmann dissertation does not disclose a set of the "ambisense" pol I-pol II viral plasmids capable of reconstituting an infectious influenza virus in the absence of a helper virus, but merely describes single pol I-pol II plasmids carrying heterologous reporter genes such as CAT and GFP. In contrast to the Examiner's assertion provided at pages 6 and 7 of the Office Action, transcription of a single reporter mRNA and expression of a reporter protein using host cell transcription/translation machinery or replication of a single reporter RNA in the presence of viral polymerase proteins supplied by a helper virus (FPV, pp. 114-115 in the Hoffmann dissertation) does not provide any teaching or suggestion of how all wild-type viral RNAs can be transcribed/replicated using pol I-pol II plasmids to achieve successful generation of infectious viral particles. In fact, the Hoffmann dissertation does not even provide a suggestion of making a single pol I-pol II plasmid encoding a single viral gene segment, much less a full set of such plasmids.

Applicants respectfully disagree with the Examiner's statement at page 6 of the Office Action that "the plasmid of Hoffmann does not require helper virus". Applicants respectfully note that it is only pol II-mediated CAT mRNA and protein production that does not require the helper virus, while expression of CAT mRNA from cRNA generated from vRNA produced from an "ambisense" pol I-pol II plasmid does. In light of the clear need for helper virus to obtain CAT mRNA of vRNA, there is no basis to generate even a single "ambisense" plasmid containing an influenza virus segment, much less a full set of such plasmids.

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[2] Applicants respectfully note that this statement directly contradicts the Examiner's statement at page 5 of the Office Action, which argues that, based on the review by Pekosz *et al.*, one would desire a helper-free system that had fewer plasmids but the efficiency of the Neumann et al. 17-plasmid system.

Furthermore, as is well known in the art and disclosed in the Neumann *et al.* article (see, *e.g.*, p. 9347 left col., ¶3), the specific amount of each of the viral proteins encoded by a set of plasmids is critical for successful production of the infectious virus. It follows that transcription/replication of a single reporter-encoding pol I-pol II plasmid disclosed in the Hoffmann dissertation provides no suggestion of how to successfully achieve appropriate levels of expression of each of the viral proteins in order to reconstitute an infectious viral particle. At pages 6-7 of the Office Action, the Examiner argues that optimizing unequal levels of viral protein expression is a routine task. It may be a routine task when the plasmids for protein expression and replication of genomic segments are provided separately, as in Neumann *et al.* It is by no means routine when they are part of the same plasmid construct.

The scientific literature provides further evidence that the claimed invention was not predictable. Hoffmann *et al.* (Virology, 2000, 267:310-7) describes reconstituting an infectious virus by introducing a single pol I-pol II plasmid encoding an influenza PB1 protein in the system of Neumann *et al.* Such "routine" achievement would not have merited a separate publication in this highly regarded peer-reviewed journal if it were, in fact, routine. Thus, Hoffmann *et al.* publication is a clear proof that the showing of the ability of a single pol I-pol II plasmid encoding an influenza PB1 protein to properly function during the reconstitution of an infectious virus in the absence of a helper virus (when all other viral proteins and vRNAs were encoded by pol I-only and pol II-only plasmids of Neumann *et al.*), was considered a significant and non-obvious advancement in the field in view of Neumann *et al.* and the Hoffmann dissertation. Reconstitution of an infectious virus in the absence of a helper virus using only pol I-pol II plasmids encoding all influenza viral segments is a more complex and unpredictable, and thus less obvious advancement. This was not achieved even in Hoffmann *et al.* publication, but was only achieved in the present invention.

In sum, the single reporter-encoding pol I- pol II plasmid described in the Hoffmann thesis does not provide a reasonable expectation of success with respect to expression of wild-type virus genes and does not provide sufficient expectation of success to attempt the expression of all viral components in a cell using the pol I-pol II system, much less successfully achieving the difficult goal of reconstituting an infectious virus. The Neumann *et al.* article does not teach the pol I-pol II system, but teaches separate pol I-only and pol II-only plasmids and the desirability of keeping them separate. Nothing in Neumann suggests the desirability of substituting the pol I-only and pol II-only plasmids for Hoffmann's pol I-pol II system, nor does anything in Hoffmann provide a basis for using the pol I-pol II plasmids in the Neumann plasmid-based system.

In other words, neither the Hoffmann dissertation nor Neumann *et al.* article provide any expectation of success or suggestion to be combined with the other reference or to modify the disclosed compositions, so that they in any way suggest a single "ambisense" pol I-pol II plasmid encoding a viral gene segment, much less the claimed composition of a set of "ambisense" pol I-pol II plasmids for the generation of infectious influenza viruses from cloned viral cDNA and related methods encompassed by the present claims.

The actual teachings of the references taken as a whole do not suggest the claimed invention, and the rejection requires impermissible hindsight reconstruction of various unconnected bits and pieces of the references to sustain itself. It is well settled however, that such hindsight reconstruction is an error. The courts have held that it is improper to use hindsight to combine elements found in the prior art to arrive at a determination of obviousness. *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1532 (Fed. Cir. 1988); *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992); *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1552 (Fed. Cir. 1983). The Examiner must show some objective teaching from the art that would lead an individual to combine the references, *i.e.*, there must be motivation. In particular, the mere

fact that the teaching of a reference may be modified in some way so as to achieve the claimed invention does not render the claimed invention obvious unless the prior art suggested the desirability of the modification (emphasis added). See *Fritch, supra* and *Ex parte Obukowicz*, 27 USPQ2d 1063 (Bd. Pat. App. & Intf. 1993).

However, as specified above, even if improperly combined, Neumann *et al.* and the Hoffmann dissertation do not disclose or suggest the present invention. The existence of a later Hoffmann *et al.* publication (Virology, 2000, 267:310-7) is a clear proof of non-obviousness of introducing even a single pol I-pol II plasmid in the system of Neumann *et al.*

Commercial Success of the Invention

Applicants further note that the non-obviousness of the plasmid composition recited in the present claims is also demonstrated by its projected commercial success due to faster generation of reassorted viruses which makes it possible to shorten the time to get new vaccines to the market. At pages 7-8 of the Office Action, the Examiner states that the applicants have not provided convincing evidence of commercial success. In response, applicants note that the very fact that the invention has been already licensed to MedImmune, Inc., one of the leading companies in the field of combating infectious diseases and specifically influenza (see corporate profile attached as Exhibit B[3]), is a clear evidence of projected commercial success.

Applicants respectfully disagree with the Examiner's statement at page 8 of the Office Action that "long felt need is not an issue because influenza vaccines are in wide use". Applicants note that, in contrast to the Examiner's assertion, predictable generation of new virus reassortments against the seasonal virus strains is a great advance. As further specified in the recent review article by Webby and Webster (Science, 2003, 302:1519-1522, attached as Exhibit

[3] Applicants respectfully note that MedImmune Vaccines has as its lead product FluMist™, the first influenza vaccine delivered as a nasal mist to be commercially available in the United States.

C), while the traditional reassortant approach can be used to produce vaccines to standard influenza viral strains, it cannot be used to generate a sufficient amount of vaccine during a pandemic or to address the long felt need to produce a safe vaccine to strains (*e.g.*, H5 and H7 subtypes) that have caused pandemics in past years and whose hemagglutinin and/or neuraminidase surface glycoprotein genes must first be modified before they can be safely used to generate a vaccine (see the paragraph bridging pages 1520-1521). As stated in the second paragraph at page 1521 of the Webby and Webster review article, the use of plasmid-based reverse genetic systems constitutes the most promising means to produce influenza vaccines during pandemics. Figure 2 of this article (see also the description of the figure) explains the use of the plasmid system of the present invention (referred to therein as the "reverse genetics system") to produce a pandemic influenza vaccine.

In light of the foregoing legal considerations and arguments, it is respectfully submitted that pending claims are not obvious over the cited art. Reconsideration and withdrawal of the obviousness rejection is believed to be in order.

CONCLUSION

Applicants request entry of the foregoing amendments and remarks in the file history of this application. In view of the above amendments and remarks, it is respectfully submitted that claims 2-5, 7-17, 19-29, 32, 39, and 42-45 are now in condition for allowance and such action is earnestly solicited. If the Examiner believes that a telephone conversation would help advance the prosecution in this case, the Examiner is respectfully requested to call the undersigned agent

at (212) 527-7634. The Examiner is hereby authorized to charge any additional fees associated with this response to our Deposit Account No. 04-0100.

Respectfully submitted,

Dated: January 12, 2004

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INTERVIEW SUMMARY

All participants (applicant, applicant's representative, PTO personnel):

(1) M Hill ; J Stucker (3) J Klein-Evans

(2) Phil Felner (4) E Hoffman

Date of Interview

Type: ☐ Telephonic ☒ Personal (copy is given to ☒ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☒ No If yes, brief description:

Agreement ☐ was reached. ☒ was not reached.

Claim(s) discussed: all pending

Identification of prior art discussed: all

Description of the general nature of what was agreed to if an agreement was reached, or any other comments:

- agreed on w fixing 112 1st. + 102(e). *

- talked about 103 - issues of minimum plasmids + unpredictable result of using plasmids

* 112 1st - fix by limiting claims to Influenza

* 102(e) fix by Katy

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

1. ☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

Unless the paragraph above has been checked to indicate to the contrary. A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a response to the last Office action has are ready been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW.

2. ☐ Since the Examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action. Applicant is not relieved from providing a separate record of the interview unless box 1 above is also checked.

Examiner Note: You must sign this form unless it is an attachment to another form.

Investor Summary
- Real-Time Quote
- Historical Stock Price
- Price Lookup
- News Releases
- Annual Report
- SEC Filings
- Section 16 Reports
- Earnings Estimates
- Fundamentals

Financial Events
- Events Calendar
- Conference Call Webcasts

Corporate Overview
- History
- Management
- Community Involvement
- Strategic Alliances
- Analysts
- Literature Request
- Investor FAQs

Contact Information

Email Alerts

Corporate Overview

MedImmune, Inc. is a fully integrated biotechnology company founded in 1988. It is focused on developing and marketing products that address medical needs in areas such as infectious disease, immune regulation, and cancer. The company's capabilities span the entire spectrum of pharmaceutical development, including discovery research, clinical development, data analysis, regulatory affairs, quality control, manufacturing, and sales and marketing.

MedImmune is headquartered in Gaithersburg, Maryland and employs over 1,500 people. The company has additional operations in Frederick, Maryland; Philadelphia, Pennsylvania; Mountain View, California; Speke, the United Kingdom; and Nijmegen, the Netherlands. The company also has approximately 210 sales representatives strategically located throughout the United States.

MedImmune's oncology focused subsidiary, MedImmune Oncology, Inc. is responsible for the clinical development and marketing of our cancer-focused products, including Ethyol® (amifostine) and VitaxinT . This subsidiary was created through the acquisition of U.S. Bioscience, a cancer-focused company, in November 1999.

In January 2002, MedImmune acquired Aviron (now MedImmune Vaccines), a vaccine company located in Mountain View, California. Aviron's lead product was FluMistT, the first influenza vaccine delivered as a nasal mist to be commercially available in the United States.

MedImmune has formed partnerships with key sales and marketing organizations throughout the world. Abbott International is the exclusive distributor of Synagis® outside the United States. As of January 31, 2003, Abbott International had filed 58 new drug applications throughout the world and received 47 regulatory approvals. Similarly, Schering-Plough Corporation markets and distributes Ethyol® outside the U.S. As of March 2001, Ethyol had received regulatory approvals in 60 countries worldwide.

MedImmune posted record revenues and earnings in 2002, led by the commercial success of our flagship product, Synagis, the first and still the only monoclonal antibody approved by the FDA to prevent an infectious disease. During 2002, worldwide sales of Synagis grew 29 percent to $668 million from $516million in 2001. For the year ended December 31, 2002, MedImmune reported a loss of $1.1 billion or $4.40 per share. This loss reflects the impact of a $1.2 billion in-process research and development charge associated with the purchase of MedImmune Vaccines (formerly known as Aviron), as well as the inclusion of MedImmune Vaccines' operations in MedImmune's results as of January 10, 2002.

MedImmune also announced "adjusted" results for 2002, which exclude certain amounts associated with the acquisition of MedImmune Vaccines. MedImmune computes "adjusted" earnings by adding back amounts that are related to the acquisition of MedImmune Vaccines, and the company believes that these results are more indicative of the underlying trends in the operations of the business. MedImmune's adjusted earnings for 2002 were $107 million, or $0.42 per diluted share.

MedImmune currently has four core marketed products, and ten products in various stages of clinical testing. Beyond that, our research and development preclinical pipeline boasts new opportunities for future clinical evaluation. MedImmune's candidate vaccines may one day address unmet medical needs including alternative delivery of a vaccine to prevent the flu, prevention of cervical cancer caused by human papillomavirus, as well as parainfluenzavirus, cytomegalovirus, and Streptococcus pneumoniae infections. Additionally, MedImmune is developing new and improved antibody products to treat and/or prevent asthma, psoriasis, rheumatoid arthritis, RSV, and cancer.

REVIEW

Are We Ready for Pandemic Influenza?

Richard J. Webby and Robert G. Webster*

During the past year, the public has become keenly aware of the threat of emerging infectious diseases with the global spread of severe acute respiratory syndrome (SARS), the continuing threat of bioterrorism, the proliferation of West Nile virus, and the discovery of human cases of monkeypox in the United States. At the same time, an old foe has again raised its head, reminding us that our worst nightmare may not be a new one. In 2003, highly pathogenic strains of avian influenza virus, including the H5N1 and H7N7 subtypes, again crossed from birds to humans and caused fatal disease. Direct avian-to-human influenza transmission was unknown before 1997. Have we responded to these threats by better preparing for emerging disease agents, or are we continuing to act only as crises arise? Here we consider progress to date in preparedness for an influenza pandemic and review what remains to be done. We conclude by prioritizing the remaining needs and exploring the reasons for our current lack of preparedness for an influenza pandemic.

In February 2003, during a family visit to mainland China, a young girl from Hong Kong died of an unidentified respiratory illness. After returning to Hong Kong, both her father and brother were hospitalized with severe respiratory disease, which proved fatal to the father. When H5N1 (avian) influenza virus was isolated from both patients, the World Health Organization (WHO) went to pandemic alert status (*1*). At about the same time, there were rumors of rampant influenza-like disease in China. Influenza experts feared that H5N1 influenza virus had acquired the ominous capacity to pass from human to human. That outbreak is now known to have been SARS, caused by a novel coronavirus.

In March 2003, another alarming situation arose on the other side of the world. A highly pathogenic H7N7 avian influenza outbreak had recently erupted in the poultry industry of the Netherlands (*2*), and workers involved in the slaughter of infected flocks contracted viral conjunctivitis. The H7N7 virus isolated from these patients had several disquieting features: Not only could it replicate in the human conjunctiva, but there was also evidence of human-to-human spread. Nearby herds of swine (which are often implicated in the adaptation of influenza viruses to humans) also showed serologic evidence of exposure (*2*). When a veterinarian died of respiratory infection (*2–5*), WHO again acknowledged the presence of a severe threat (*6*).

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Luckily, the worst-case scenarios did not come about in either of the 2003 avian influenza virus scares. However, the year's events eliminated any remaining doubts that global advance planning for pandemic influenza is necessary. They also highlighted how far, as a scientific community, we have come since the 1997 event: We are now much better equipped with technologies and reagents to rapidly identify and respond to pandemic influenza threats. On the other hand, the legislative and infrastructure changes needed to translate these advances into real public health benefits are alarmingly slow.

The Role of WHO in Influenza Surveillance and Control

In 2001, WHO initiated the development of a Global Agenda for Influenza Surveillance and Control. Its four main objectives are to strengthen influenza surveillance, improve knowledge of the disease burden, increase vaccine use, and accelerate pandemic preparedness (*7*). In May 2002, this document was adopted after proposals and public comment were invited. The document advocates the development of methods and reagents that can be used to rapidly identify all influenza virus subtypes, thereby allowing integrated influenza surveillance in humans and in other animals. WHO, with its global influenza network of more than 100 laboratories and its distinguished record of planning for yearly interpandemic influenza, is ideally situated to play a broader role in facilitating international cooperation for the rapid exchange of viruses, reagents, and information. Influenza continually evolves at the human–lower animal interface and thus can be unpredictable. As an example, within a brief period, the H7N7 virus events occurred in European poultry and humans, H5N1 viruses infected Asian poultry and humans, and novel, rapidly spreading reassortant viruses were isolated in swine in the United States (*8*, *9*). Therefore, the capacity to simultaneously manage multiple potential pandemic situations is important. The WHO global agenda document will help to prioritize areas of influenza research and facilitate national pandemic preparedness plans.

Prioritization of Viral Subtypes for Surveillance and Control

Influenza experts agree that another influenza pandemic is inevitable and may be imminent (Fig. 1). A major challenge in controlling influenza is the sheer magnitude of the animal reservoirs. It is not logistically possible to prepare reagents and vaccines against all strains of influenza encountered in animal reservoirs, and therefore, virus subtypes must be prioritized for pandemic vaccine and reagent preparation. Preliminary findings have identified the H2, H5, H6, H7, and H9 subtypes of influenza A as those most likely to be transmitted to humans. [Influenza viruses are typed according to their hemagglutinin (H) and neuraminidase (N) surface glycoproteins.] The influenza A subtypes currently circulating in humans, H1 and H3, continue to experience antigenic drift. That is, their antigenic surface glycoproteins are continually modified, allowing them to escape the population's immunity to the previous strain and thus to continue causing annual outbreaks. Although these continual modifications may lead to an increase in virulence, the mildness of the past three influenza seasons suggests that the dominance of the H1N1 and H3N2 viruses is waning as their ability to cause serious disease becomes increasingly attenuated. H2 influenza viruses are included in the high-risk category because they were the causative agent of the 1957 "Asian flu" pandemic and were the only influenza A subtype circulating in humans between 1957 and 1968. Counterparts of the 1957 H2N2 pandemic virus continue to circulate in wild and domestic duck reservoirs. Under the right conditions (which are still not completely understood), H2N2 viruses could again be transmitted to and spread among humans, none of whom under the age of 30 years now has immunity to this virus. Seroarchaeology data from the late 19th and early 20th centuries indicate that only the H1, H2, and H3

influenza virus subtypes have been successfully transmitted among humans. It is possible, but unlikely, that they are the only subtypes able to do so.

Not only are the H1, H2, and H3 influenza viruses of concern, but the H5 subtype has threatened to emerge as a human pandemic pathogen since 1997, when it killed 6 of 18 infected humans. Before that event, the receptor specificity of avian influenza viruses was thought to prevent their direct transmission to humans. Transmission from aquatic birds to humans was hypothesized to require infection of an intermediate host, such as the pig, that has both human-specific (α2-6 sialic acid) and avian-specific (α2-3 sialic acid) receptors on its respiratory epithelium. The 1997 H5N1 event demonstrated that domestic poultry species may also act as intermediate hosts. H5N1 viruses continue to emerge and evolve despite heroic measures taken to break their evolutionary cycle in the live poultry markets of Hong Kong: the elimination of live ducks and geese (the original source), the elimination of quail (the source of the internal genes of H5N1/97), and the institution of monthly "clean days," when all 1000-plus retail markets are emptied and cleaned.

Two things have become clear. Live poultry markets are potential breeding grounds for influenza and other emerging disease agents, and there is an Asian source of H5N1 influenza viruses outside of Hong Kong SAR. Between 1997 and 2003, H5N1 virus was isolated from duck meat imported from China into Korea (*10*) and Japan (*11*). These observations suggest that ducks and possibly other avian species in mainland China are a reservoir of H5N1, although there have been no official reports of H5N1 virus in China.

At the beginning of the SARS outbreak, China missed an opportunity to show the world its considerable intellectual and scientific potential (*12*). In the case of H5N1 influenza, a pandemic in waiting, it remains to be seen whether China will show leadership in proactively addressing the problem. Concerted national and international efforts are required to deal effectively with the threat.

The third virus subtype on the most wanted list is H7. The H7 and H5 viruses have a unique ability to evolve into a form highly virulent to chickens and turkeys by acquiring additional amino acids at the hemagglutinin (HA) cleavage site (HA cleavage is required for viral infectivity) (*13*). The highly pathogenic H7N7 influenza viruses that were lethal to poultry infected the eyes of more than 80 humans and killed one person (*14*). In the case of this outbreak, the Netherlands' policy of openness was important in reducing the potential threat and should serve as a model. When the virus was first detected at the end of February 2003, the European Community and international community, via the Office International des Epizooties, were notified so that surrounding countries, including Belgium and Germany, could immediately respond if the disease was detected. Culling of all poultry on infected farms and quarantine of surrounding farms succeeded in eradicating the virus once the etiologic agent was identified. After human infection was observed, an anti-influenza drug was given as prophylaxis, and vaccination with the current human influenza vaccine was done to reduce the likelihood that the avian virus would reassort with human H1N1 and H3N2 strains.

The remaining two viral subtypes on the priority list, H6 and H9, do not share the virulent phenotypes of the H5 and H7 viruses, but still pose a considerable threat. Both of these influenza viruses have spread from a wild aquatic bird reservoir to domestic poultry over the past 10 years. H9N2 viruses have also been detected in humans and in pigs (*15*, *16*) and have acquired human-like receptor specificity (*17*). Neither of these viruses was able to infect chickens before the mid-1980s. Now, for unknown reasons, H9 viruses are endemic in chickens in Eurasia and H6 viruses are becoming endemic in both Eurasia and the Americas. These facts highlight the continuing adaptation of influenza viruses in the aquatic bird reservoirs to domestic chickens.

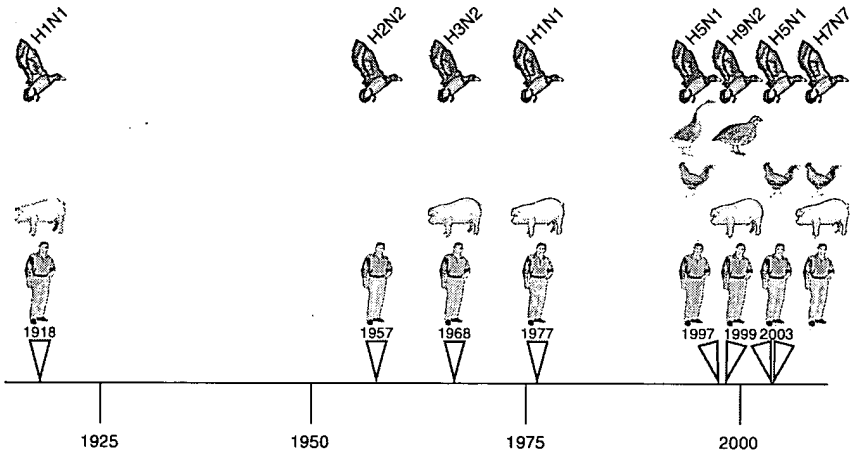

Fig. 1. Timeline of human influenza over the past 100 years. The black triangles represent documented human influenza A infections characterized by multiple cases. In each instance the species of animals implicated in the emergence of disease is highlighted. Since 1997 there has been a disproportionate increase in the number of reports of novel subtypes in humans and in the number of animal and bird species involved, suggesting that the next influenza pandemic is imminent.

The Challenge of Developing Candidate Vaccines

If the next influenza pandemic were to begin tomorrow, inactivated vaccines would offer the only immediate means of mass prophylaxis, yet their supply is limited by inadequate production capabilities and suboptimal utilization of adjuvants (*18*, *19*). The stocks of antiviral drugs are too low to cope with an epidemic and would be quickly depleted (*19*). Tissue culture–based and live attenuated vaccines are now licensed in some countries, and could supplement the supply of inactivated vaccine. Further development of these options is urgently needed to provide alternative substrates in the face of a pandemic.

Since the 1970s, influenza vaccines have been made by exploiting the tendency of the segmented influenza genome to reassort (*20*). This natural process has been used to produce vaccine strains that simultaneously contain gene segments that allow them to grow well in eggs and gene segments that produce the desired antigenicity. Natural reassortment is allowed to occur in embryonated chicken eggs, and reassortants with the desired characteristics are selected. These recombinant vaccine strains contain the hemagglutinin and neuraminidase genes of the target virus (encoding glycoproteins that induce neutralizing antibodies); their remaining six gene segments come from A/Puerto Rico/8/34 (H1N1), which replicates well in eggs and is safe for use in humans (*21*). These "6+2" reassortants are then grown in large quantities in embryonated chicken eggs, inactivated, disrupted into subunits, and formulated for use as vaccines. Although this process creates an effective and safe influenza vaccine, it is too time-consuming and too dependent on a steady supply of eggs to be reliable in the face of a pandemic emergency. Even during interpandemic periods, 6 months is required to organize sufficient fertile chicken eggs for

annual vaccine manufacture (*22*), and the preparation of the desired "6+2" recombinant vaccine strain can be a time-consuming process. Influenza vaccine preparation is seasonal and is a remarkable achievement, in that an essentially new vaccine is made every year. However, two of the viruses of greatest concern, those of the highly pathogenic H5 and H7 subtypes, cannot be successfully grown in eggs. Their unique ability to accumulate multiple basic amino acids at the site of hemagglutinin cleavage increases their ability to spread systemically in an infected host and cause significant disease (*13*). This feature also renders H5 and H7 viruses rapidly lethal to chicken embryos.

The most promising means of expediting the response to pandemic influenza is the use of plasmid-based reverse genetic systems to construct influenza virions and vaccines. These systems also offer a successful alternative means of producing H5 and H7 vaccine seed strains. Because viable viruses can be generated from individually cloned cDNA copies of each of the eight viral RNA segments, reassortment can be prospectively defined and directed, and the extra amino acids at the HA cleavage site (which are associated with high virulence) can be removed to allow rapid generation of a vaccine seed strain in eggs. Plasmids encoding the internal genes of the base vaccine are already available. A vaccine seed strain can be created by cloning the appropriate hemagglutinin and neuraminidase genes from the target virus, altering its HA connecting peptide if necessary, and transfecting an appropriate cell line (Fig. 2). This technology has been shown to be effective for the production of reassortants carrying several different surface glycoprotein combinations, including those considered to have a high pandemic potential (*23–26*). The next step is to take these plasmid-derived influenza vaccines through clinical trials to address crucial questions such as number and quantity of doses and the role of adjuvants. Most of the vaccines derived after the 1997 H5N1 episode by various alternative strategies induced a disappointing immune response (*27*). The optimal pandemic vaccination regimens can be anticipated only by collecting the necessary data and experience through clinical trials of vaccines against different subtypes of influenza virus.

Although they are well suited to the manufacture of inactivated influenza vaccines, reverse genetic systems introduce new variables. One of the most limiting of these is the need to use cell lines. There are surprisingly few suitable accredited cell lines and cell banks available, and many of those are the property of pharmaceutical companies. The practical options are very few, in view of the technical and regulatory restrictions. Perhaps the only cell line that meets all criteria for international use at this time is the African green monkey kidney cell line, Vero. However, although Vero cell lines are in widespread laboratory use, only those that are derived from WHO-approved sources and have a detailed history are acceptable for manufacture of human pharmaceuticals. A second new variable is the use of a genetically modified virus seed strain. Because the traditional vaccine strains are made by natural reassortment, they have escaped being labeled "genetically modified." This difference, although largely semantic, may affect the acceptance of the new vaccines. Before many of these traits can be tested, the virus must be amplified, inactivated, purified, and formulated for vaccine use (*22*).

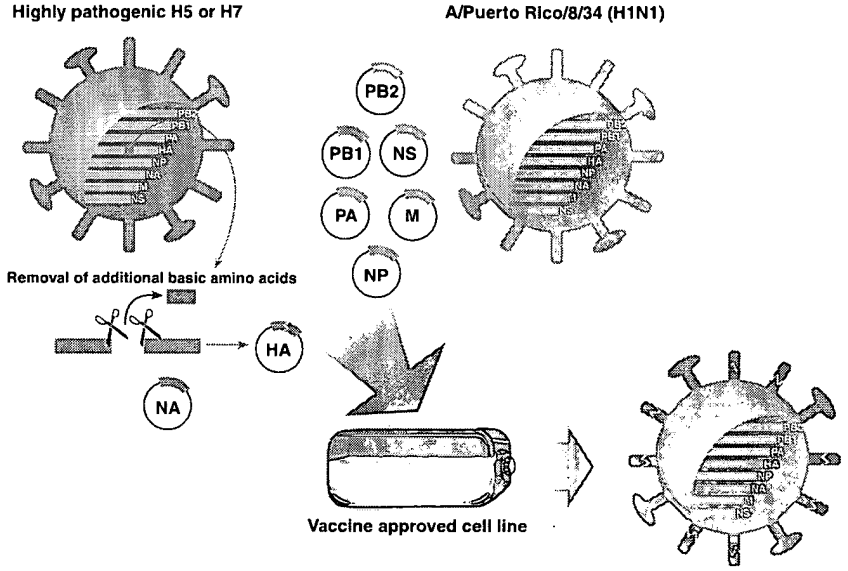

Fig. 2. Proposed method of influenza vaccine seed virus production using the eight-plasmid reverse genetics system (*23*). The hemagglutinin (HA) and neuraminidase (NA) genes from the target strain are cloned into the bacterial plasmid vector pHW2000 in a process that allows for the alteration of the HA cleavage site when necessary (see text for explanation). These two plasmids, along with six others containing the remaining influenza A gene segments derived from the master vaccine strain A/Puerto Rico/8/34 (H1N1), are then introduced into a suitable cell line (e.g., Vero). After expression of positive- and negative-sense RNA and viral proteins from these plasmids, a productive replication cycle is initiated and viable virus particles are produced.

In preparing for a pandemic threat, collaboration between government, industry, and academia is needed to overcome the obstacles and guarantee the most rapid production of a vaccine candidate. The recent SARS episode has shown that international collaboration in the face of a truly global threat is indeed possible.

The Safety Testing of Candidate Pandemic Vaccines and Liability Issues

Unfortunately, there are only a few facilities available to carry out safety testing under the high-level biocontainment conditions required for handling highly pathogenic influenza viruses. Overcoming the technical hurdles to efficient vaccine production is only the start of a long, expensive process. Manufacturing scale-up presents its own problems, not least because plant workers will have no immunity to the pathogens they will be handling. Of prime importance is vaccine safety testing, but the need for safety testing will have to be balanced against the need for rapid mass production of a vaccine. In response to the 2003 H5N1 scare in Hong Kong, WHO has created an Interim Biosafety Risk Assessment (*28*) guideline for the safety testing of pandemic vaccines, particularly the H5 and H7 subtypes, signifying a substantial advance in preparedness for the production of a pandemic influenza vaccine.

A major risk for all vaccine manufacturers is the occurrence of adverse reactions in a percentage of recipients. These reactions may be attributable to the vaccine, to the host, or (most likely) to a unique combination of the vaccine and the host genetic factors. Guillain-Barré syndrome in human beings first became apparent during the U.S. swine influenza vaccination program (*29*, *30*). The inevitability of adverse reactions underscores the product liability dilemma inherent in any vaccine program. The risk of devastating financial liability, and the unavailability or high cost of liability insurance, are increasingly discouraging vaccine manufacture, especially for universal use.

Legislative measures can be taken to reduce the impact of liability exposure. For example, the U.S. Congress passed the National Childhood Injury Compensation Act of

1986 (the "Vaccine Act"), which created a no-fault compensation program funded by an excise tax on vaccines. Plaintiffs need only establish that their injuries were caused by the vaccine. Claimants who are not satisfied with the administrative decision may still elect to sue the manufacturer, but the legal arguments available to the claimant are limited. Although the Vaccine Act represents progress in achieving a balance between consumer and manufacturer concerns, it would not apply to vaccines given to the general population, such as those for influenza or smallpox. Congress again attempted to address these concerns in a provision of the Homeland Security Act of 2002, and an Institute of Medicine panel is currently wrestling with the problem as well; however, drug manufacturers remain hesitant. The bottom line is that unless the government authorities of every country implement mechanisms that equitably limit vaccine liability, no prospective vaccine for H5N1, H7N7, or any other threatening influenza virus is likely to be produced for universal human use. It is hoped that governments will rise to the occasion after a crisis emerges, but logic suggests that the issue should be addressed now.

Antiviral Drugs

A global influenza strategy would call for the stockpiling of influenza antiviral drugs for use in the event of a pandemic until vaccines can be prepared. "But," as noted by Albert Osterhaus (*31*), "no country has yet started to stockpile antiviral drugs." The potential value of antivirals was demonstrated in the recent H7N7 outbreak in poultry and humans. Further, because epidemiological modeling has suggested that it is more infectious than SARS (*32–34*), influenza is unlikely to be controllable by SARS-like quarantine measures. The estimated 10 billion U.S. dollar cost of SARS and the societal disruption it caused in China and Toronto make a compelling case for stockpiling of antiviral drugs.

Pandemic influenza has already threatened twice in 2003. The events associated with these outbreaks show that we are in a much better position to rapidly respond to an influenza threat than we were in 1997; however, much remains to be accomplished. Overall, our state of preparedness is far from optimal.

Priorities to Ensure Pandemic Preparedness

To conclude, let us revisit our concern that the next influenza pandemic alert may involve a virus that has acquired the capacity to spread from human to human. What are our most urgent needs?

1) A sufficiently large supply of anti-influenza drugs to reduce the severity and spread of infection. Specific efficacious drugs are available, but no country has yet invested in stockpiling.

2) A vaccine matching the subtype of the emerging pandemic influenza strain that has been tested in clinical trials and for which manufacturers are prepared to "scale up" production. Such a vaccine would probably not match the emerging strain antigenically and would not prevent infection, but it could reduce the severity of illness until a matching vaccine is produced. Such vaccines have been discussed for 20 years. None is available, but specific plans to produce such a vaccine are currently being formulated.

3) The preparation, testing (safety and clinical trials), and availability of a vaccine derived by reverse genetics. The scientific technology is in place to achieve this goal, but manufacturing, intellectual property, and liability issues remain unresolved. In the event of a pandemic, reverse genetics would be the most rapid means by which to produce an antigenically matched vaccine. To be truly prepared, such a vaccine needs to be produced and tested now to identify and resolve the issues, rather than doing so in direct response to an emergency.

4) An improvement in the global influenza vaccine manufacturing capacity. Without the use of adjuvants, the current capacity is inadequate and could not be quickly augmented. The country best prepared to meet this need is Canada; in Ontario, influenza vaccination is recommended and available at no charge to people of all ages during the influenza season (*35*). This progressive strategy during interpandemic years will ensure the vaccine-manufacturing capacity of that region.

The conclusion of this analysis is inescapable: The world will be in deep trouble if the impending influenza pandemic strikes this week, this month, or even this year. It is now time to progress from talking about pandemic vaccines to taking action. Our hope is that the "Ontario experiment" will inspire other regions of the world to similarly promote the expansion of manufacturing capacity for influenza vaccines.

Although reverse genetics offers great advantages for the rapid preparation of influenza vaccine strains and for understanding pathogenesis (*36*), the reverse side of this benefit is its potential for the development of bioterrorism agents (*37*). Regardless of human endeavors, nature's ongoing experiments with H5N1 influenza in Asia and H7N7 in Europe may be the greatest bioterror threat of all. The time for talking is truly over. We must be prepared.

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